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Allopolyploidy – a shaping force in the evolution of wheat genomes

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Abstract. Recent studies have shown that allopolyploidy accelerates genome evolution in wheat in two ways: (1) allopolyploidization triggers rapid genome changes (revolutionary changes) through the instantaneous generation of a variety of cardinal genetic and epigenetic alterations, and (2) the allopolyploid condition facilitates sporadic genomic changes during the life of the species (evolutionary changes) that are not attainable at the diploid level. The revolutionary changes comprise (1) non-random elimination of coding and non-coding DNA sequences, (2) epigenetic changes such as DNA methylation of coding and non-coding DNA leading, among others, to gene silencing, (3) activation of genes and retroelements which in turn alters the expression of adjacent genes. These highly reproducible changes occur in the F_1 hybrids or in the first generation(s) of the nascent allopolyploids and were similar to those that occurred twice in nature: first in the formation of allotetraploid wheat (~ 0.5 million years ago) and second in the formation of hexaploid wheat (~ 10,000 years ago). Elimination of non-coding sequences from one of the two homoeologous pairs in tetraploids and from two homoeologous pairs in hexaploids, augments the differentiation of homoeologous chromosomes at the polyploid level, thus providing the physical basis for the diploid-like meiotic behavior of allopolyploid wheat. Regulation of gene expression may lead to improved inter-genomic interactions. Gene inactivation brings about rapid diploidization while activation of genes through demethylation or through transcriptional activation of retroelements altering the expression of adjacent genes, leads to novel expression patterns. The evolutionary changes comprise (1) horizontal inter-genomic transfer of chromosome segments between the constituent genomes, (2) production of recombinant genomes through hybridization and introgression between different allopolyploid species or, more seldom, between allopolyploids and diploids, and (3) mutations. These phenomena, emphasizing the plasticity of the genome with regards to both structure and function, might improve the adaptability of the newly formed allopolyploids and facilitate their rapid and successful establishment in nature.

Allopolyploidy (= amphiploidy) is an evolutionary process whereby two or more different genomes are brought together into the same nucleus by inter-specific or inter-generic hybridization followed by chromosome doubling. Allopolyploidy is prevalent among many groups of plants (Manton, 1950; Stebbins, 1950, 1971; Grant, 1971). Several important crop plants, such as bread and durum wheat, oat, cotton, canola, coffee, and tobacco, are allopolyploids. Recent DNA sequencing indicates that also the classical diploid maize is in fact an ancient allopolyploid (paleoallopolyploid) that underwent massive diploidization (Helentjaris et al., 1988; Gaut and Doebley, 1997; Gaut, 2002). It is quite possible therefore, that many more diploids are paleoallopolyploids. Hence, allopolyploidy, perhaps more than any other process, has played a major role in the origin of many species and thus has driven and shaped the evolution of vascular plants.

The widespread occurrence of allopolyploidy is attributed to the potential of allopolyploid species to adapt to a wide range of ecological niches and survive better in unstable environments than their diploid progenitors (Stebbins, 1950, 1971; Grant, 1971; see also several reviews in the book on Polyploidy, edited by Lewis, 1980). Allopolyploids are characterized by a diploid-like meiotic behavior (exclusive bivalent pairing of homologous chromosomes) that brings about full fertility, disomic inheritance and stabilization of the hybrid condition due to the prevention of segregation of homoeoalleles (alleles of orthologous

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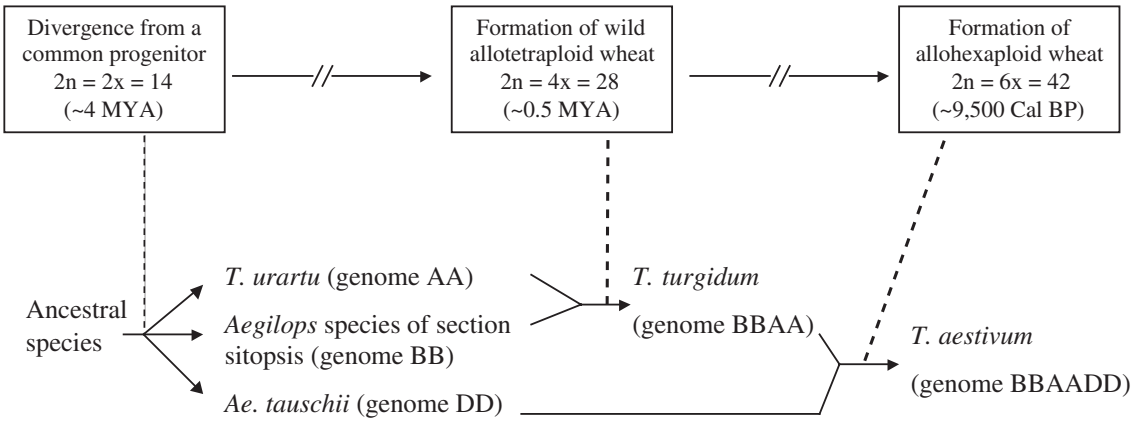

Fig. 1. Origin of allopolyploid wheat.

genes). This genetic system, reinforced, in many cases, by predominant self-pollination, leads to true breeding and permanent maintenance of inter-genomic favorable genetic interactions. The duplicated genetic material facilitates tolerance of genomic alterations that would be fatal at the diploid level and, on the other hand, can bring about accelerated evolution at the polyploid level. Consequently, allopolyploid species are evolutionary very successful; they are aggressive, efficient colonizers and compete well with their diploid progenitors. In most cases they distribute in a larger geographical area, occupy more versatile habitats, and exhibit wider ranges of morphological, biochemical and molecular variation than their diploid parental species.

Recent molecular studies in several allopolyploid plant species have shown that the allopolyploidization process induces a variety of cardinal genomic changes that are not attainable at the diploid level. Some of these genomic changes are presumably crucial for the construction of an efficient genetic system facilitating the establishment in nature of the newly formed allopolyploids and for the rapid build up of a wealth of genetic variability. These effects were described in several recent reviews (Leitch and Bennett, 1997; Comai, 2000; Soltis and Soltis, 2000; Wendel, 2000; Pikaard, 2001; Levy and Feldman, 2002, in press). The review here will focus mainly on the effect of allopolyploidy on the genomes of wheat – a genus that has contributed one of the best models to the study of allopolyploidy. It will review the accumulating evidence of allopolyploidy as a trigger and/or facilitator for accelerated genome evolution in this genus, immediately after allopolyploidization or during the life of the allopolyploid species. Overall, we will try to learn from the wheat case, what are the causes for the formidable evolutionary success of allopolyploidy in nature.

Wheat – a classical example of evolution through allopolyploidy

Schultz (1913) assembled the first natural classification of wheat by dividing the genus *Triticum* into three major taxonomic groups: einkorn, emmer, and dinkel. This classification was supported by the pioneering cytological study of Sakamura (1918), who found that Schultz's three wheat groups also differ in their chromosome number; the einkorns are diploids (2n = 2x = 14), the emmers are tetraploids (2n = 4x = 28), and the dinkels are hexaploids (2n = 6x = 42). Soon after, the studies of Kihara (1919, 1924) and Sax (1927) on chromosome pairing in hybrids between species of different ploidy levels showed that 7 chromosome pairs of diploid wheat (genome A) plus 7 additional pairs (genome B) constitute the 14 pairs of tetraploid wheat. These 14 pairs plus additional 7 pairs (genome D) make up the 21 pairs of hexaploid wheat. Thus, the various wheat species comprise an allopolyploid series based on x = 7 (Fig. 1; reviewed by Feldman, 2001). Similar studies, summarized by Kihara (1954), showed that also all the polyploid species of the closely related genus, *Aegilops*, are allopolyploids. The allopolyploid species of *Aegilops* and *Triticum* behave like typical genomic allopolyploids, i.e., their chromosomes pair in a diploid-like fashion and the mode of inheritance is disomic. Because of the obvious importance of the allopolyploid (bread and durum) wheat for human nutrition, their cytogenetic structure and origin have been intensively investigated. However, during the last 80 years the cytogenetic and evolutionary studies of allopolyploid wheat have mainly focused on the origin of these allopolyploid species rather than on their mode and pattern of evolution.

Studies on the origin of wheat showed that the A and D genomes of allopolyploid wheat share a high degree of homology with the diploid genomes of *T. urartu* (2n = 2x = 14; genome AA) and *Aegilops tauschii* (= *Ae. squarrosa*; 2n = 2x = 14; genome DD), respectively. Indeed, conclusive evidence that the D genome of hexaploid wheat was donated by *Ae. tauschii* was provided by McFadden and Sears (1944, 1946) and Kihara (1944), who independently, produced synthetic hexaploids from crosses of tetraploid wheat with *Ae. tauschii* that resembled certain natural hexaploids. Hybrids between synthetic and natural hexaploids exhibit complete chromosome pairing at meiosis and are usually fully fertile (McFadden and Sears, 1946; Kihara and Lilienfeld, 1949).

Tetraploid wheat is an older species than hexaploid wheat; it was formed about half a million years ago (Huang et al., 2002)

Table 1. Types and characteristics of genome changes in allopolyploid wheat

Revolutionary changes (triggered by allopolyploidization)	Evolutionary changes (facilitated by allopolyploidy)
Occur immediately after allopolyploidization	Occur during the life of the allopolyploid species
Genetic and epigenetic changes	Mostly genetic changes
Species specific	Population or biotype specific
Lead to diploid-like meiotic behavior (cytological diploidization)	Promote genetic diversity, flexibility, and adaptability
Improve harmonic functioning of the divergent genomes	
Stabilize the nascent allopolyploid and facilitate its establishment as a new species in nature	

while hexaploid wheat was formed only 10,000 years ago (Feldman, 2001). Therefore, the genomes of tetraploid wheat have undergone a considerable differentiation and it is more difficult to identify the diploid donor of the B genome of allopolyploid wheat than the more recent donor of the D genome. Morphological, geographical, cytological, genetic, biochemical and molecular evidence have been used to implicate one of the species of section *Sitopsis* of *Aegilops* as the donor of the B genome (reviewed in Feldman et al., 1995). From all the *Sitopsis* species, *Ae. speltoides* (2n = 2x = 14; genome SS) is the closest to the donor of the B genome. However, the progenitor of the B genome, which contributed the cytoplasm of tetraploid and hexaploid wheat, remains uncertain because a wild species with a high degree of homology to the B genome of wheat has not been found. The ambiguous nature of the B genome can be explained by one of the following possibilities: the diploid progenitor still exists but was not yet discovered; it is extinct; the diploid donor of the B genome has changed after the formation of the allotetraploid; or the B genome of wheat has rapidly evolved in the allopolyploid condition through a variety of structural changes, and particularly through introgression of chromosomal segments from other allopolyploid or diploid species.

Because of the economic importance of allopolyploid wheat, and because of the relative ease of production of amphiploids in this group, many synthetic allopolyploids were produced and their cytogenetic behavior and economic potential was evaluated. A famous case is the production of octoploid and hexaploid triticale by crossing hexaploid or tetraploid wheat, respectively, with rye. The idea was that this new crop would combine the quality of wheat with the cold hardiness and prolificacy of rye. However, the use of triticale and the other synthetic allopolyploids, in studies on the impact of allopolyploidy on genome evolution, has been very limited until recent years.

The impact of allopolyploidy on genome changes

Allopolyploidy being a revolutionary rather than an evolutionary mode of speciation is the only way through which a new species is formed in one step. Since this species is a hybrid containing two or more different genomes, allopolyploidy creates a considerable stress on the plant. Consequently, the newly formed allopolyploid faces several challenges such as the pattern of chromosome pairing, the effect of extra gene dosage, gene expression orchestration, and concerted DNA replication, already in the early stages of its formation. To meet these challenges and to ensure increased fitness and consequently, successful establishment in nature, the newly formed allopolyploid must undergo immediate genomic changes. Little information exists about the nature of genomic changes that are required for successful speciation via allopolyploidy. In particular, the mechanisms by which two or more different genomes achieve harmonic coexistence in the same nucleus (genetic diploidization) and the mechanisms causing rapid differentiation of the homoeologous chromosomes so that they will not be able to pair and recombine at meiosis (cytological diploidization), are largely unknown. While genetic diploidization mechanisms may involve gene silencing, mainly via DNA methylation or elimination of duplicate genes, or activation of genes that are usually silent at the diploid level (Liu et al., 1998b; Shaked et al., 2001; Kashkush et al., 2002; and Ozkan, Levy and Feldman, unpublished), cytological diploidization may involve elimination of DNA sequences that are presumably implicated in homology search and initiation of pairing at meiosis, from one pair of homoeologous chromosomes in tetraploids and from two pairs in hexaploids (Feldman et al., 1997; Liu et al., 1998a; Ozkan et al., 2001).

Inter-generic hybridization and chromosome doubling are two "genetic shocks" that the nascent allopolyploid may react to them in a burst of genomic changes. Indeed, recent studies in wheat (Feldman et al., 1997; Liu et al., 1997, 1998a, b; Salina et al., 2000, 2004; Ozkan et al., 2001; Shaked et al., 2001; Kashkush et al., 2002, 2003;) and in several other allopolyploid species (Song et al., 1995; Comai, 2000; Soltis and Soltis, 2000; Wendel, 2000; Pikaard, 2001, and references therein) have shown that the allopolyploidization process triggers a wide range of fundamental genomic alterations. Moreover, the allopolyploid condition, i.e., the duplication of most of the genes, facilitates many genomic changes, some of which are either unattainable or unfavorable at the diploid level. Understanding the nature, causes, mechanisms and evolutionary significance of some of these genomic changes has been the main theme of our studies in recent years. These changes, triggered or facilitated by allopolyploidy, were divided into two types: revolutionary and evolutionary (Table 1). Revolutionary changes occur immediately (already in F_1 or within one to several generations after allopolyploidization), they are genetic and epigenetic

Table 2. Revolutionary changes (occurring immediately after allopolyploidization)

	On the genome level	On the gene level
Genetic:	Elimination of low-copy DNA sequences from homoeologous chromosomes and genomes	Gene elimination
	Elimination, reduction or amplification of high-copy sequences	Inter-genomic interactions (suppression, activation, dosage compensation, concerted evolution, gene conversion)
	Inter-genomic invasion	Modification of gene function through duplications
	Chromosomal repatterning (translocations)	
	Elimination rRNA genes (nucleolar organizers)	
Epigenetic:	Changes in chromatin remodeling (conformation) due to methylation and acetylation	Gene inactivation or activation through alterations in cytosine methylation
		Differential inactivation of homoeoalleles in different tissues (semifunctionality of homoeoalleles)
		Alteration of gene expression through transcriptional activation of retroelements

changes, and, because of their reproducibility in different synthetic and natural allopolyploids with the same genomic combination, they are species specific. These changes lead presumably to diploid-like meiotic behavior, restore full fertility, and improve the harmonic functioning of the two or more diverged genomes that are included in one nucleus – a variety of changes that stabilize the nascent allopolyploid and facilitate its establishment as a new species. Evolutionary changes, on the other hand, are mostly genetic changes, occurring sporadically at random over a long time period during the life of the allopolyploid species, and therefore, they characterize population(s) or biotype(s). The evolutionary changes promote genetic diversity, flexibility and adaptability. Both types of changes lead to structural and functional changes expressed in chromosome behavior and segregation during meiosis, and in alterations in genome expression due to inactivation or activation of a large number of genes.

Revolutionary changes

The revolutionary changes (Table 2), some of which facilitate the speedy establishment of the newly formed allopolyploid, are expressed at two levels: structural (cytological diploidization) and functional (genetic diploidization).

Structural changes

It was found that elimination of non-coding, low-copy DNA sequences, that are present in all the diploid species of the genera *Aegilops* and *Triticum* but, occur in only one pair of chromosomes (chromosome-specific sequences, CSSs) or in several chromosome pairs of one genome (genome-specific sequences, GSSs), in allopolyploid wheat, happens in the very early stages of allopolyploidy (Feldman et al., 1997; Liu et al., 1998a). To study the extent and pattern and, in particular, to determine the accurate time of elimination we produced 35 different inter-specific and inter-generic F_1 hybrid combinations and 22 amphiploids between species of *Aegilops* and *Triticum* (Ozkan et al., 2001). One half of the amphiploids had genomic combinations similar to those of natural allopolyploids ("Natural") and the remainder half had genomic combinations that do not exist in nature ("Non-Natural"). Using several CSSs and GSSs as probes and various DNA restriction enzymes, including methylation sensitive and insensitive isoschizomers, and through mapping with aneuploid lines, Southern analysis of these hybrids and amphiploids and their parental plants showed that elimination from one genome occurred very rapidly and in a reproducible manner; elimination of GSSs started as soon as in the F_1 hybrid and that of CSSs in the first generation following chromosome doubling (Ozkan et al., 2001). Elimination was more frequent and rapid in "Natural" amphiploids than in the "Non-Natural" ones. To obtain an estimate of the rate of elimination, an unbiased set of sequences was analyzed by AFLP. It was found that elimination started in the F_1 hybrid, or in the first amphiploid generation, was reproducible and involved up to 15% of the genomic DNA (Shaked et al., 2001).

Also elimination of repetitive DNA sequences was found in several recent studies. Significant reduction in the copy number of *Spelt1*, a repetitive sub-telomeric sequence that represents 2% of the *Ae. speltoides* genome, was found in the first generation of amphiploids having *Ae. speltoides* as a diploid parent (Salina et al., 2000, 2004). Neither ploidy level nor the direction of the cross affected the elimination pattern in these newly-formed amphiploids. Similarly, it was found that global elimination of transposon-like highly repetitive sequences from genomes B, G, and D of allopolyploid wheat, occurred during the allopolyploidization process (Zhang et al., 2003). Also, rearrangements in repetitive DNA, such as rDNA, were observed in the early stages of wheat allopolyploid formation (Sasakuma et al., 1993). Finally, and in agreement with the above molecular data, the total nuclear DNA content of triticale (Boyko et al.,

1988) and of several wheat amphiploids (Ozkan et al., 2003) was lower than that expected for the combined genomes of their diploid progenitors.

Elimination of a given sequence was only from one genome in tetraploid and from two genomes in hexaploid, resulting in the increase of the physical divergence between homoeologous chromosomes. As a result of this elimination, every homologous pair possesses different CSSs than the other pairs in the same homoeologous group. The elimination provides the physical basis for the diploid-like meiotic behavior characterizing allopolyploid wheat. Consequently, a possible result of this elimination is the suppression of inter-genomic pairing and recombination and thus, increased fertility and stability of the hybrid condition. It is interesting to mention in this regard, that the extent of DNA elimination in "Natural" and "Non-Natural" amphiploids was positively correlated with seed fertility and negatively correlated with the amount of multivalent pairing (Ozkan H., personal communication).

The CSSs tend to cluster in several specific regions in each chromosome arm (Liu et al., 1997). Since these regions are perhaps the only homologous-specific regions, the CSSs may be involved in processes of homology recognition and pairing initiation at early stages of meiosis. Indeed, recent data (G. Grafi, B. Liu, F. Han, C. Melamed-Bessudo and M. Feldman, in preparation) indicate that chromosome-specific sequences possess polycomb response elements (PREs) that bind specifically premeiotic polycomb proteins. It is speculated (G. Grafi, B. Liu, F. Han, C. Melamed-Bessudo and M. Feldman, in preparation) that meiotic pairing is initiated by complexing the PREs of the chromosome-specific sequences with premeiotic polycomb proteins.

The prevention of inter-genomic pairing owing to the instantaneous, non-random elimination of sequences has been critical in the evolution of allopolyploid wheat. A second system involved in the exclusive bivalent pairing in allopolyploid wheat, is a genic one, consisting of the genes *Ph1* on chromosome arm 5BL and *Ph2* on chromosome arm 3DS (Sears, 1976). These genes suppress pairing of homoeologous chromosomes while allowing homologs to pair regularly. The genic system has probably evolved later, superimposing itself on and, thereby, reinforcing the already existing system of physical homoeologous differentiation. While the genic system is very effective in suppressing homoeologous pairing in inter-specific and inter-generic hybrids, its suppressive effect on homoeologous pairing in allopolyploid wheat is almost superfluous since in plants deficient for *Ph1* there is relatively very little homoeologous pairing (Sears, 1976). Interestingly, and in accord with the above, gene(s) like *Ph* were not found in all the allopolyploid species of *Aegilops* and, in spite of this, these species exhibit an exclusive bivalent pairing.

Functional changes

It is imperative to achieve, as early as possible, harmonious function of the two diverged genomes that were included in one nucleus. This can be accomplished through genetic or epigenetic control of gene expression. In wheat, inter-genomic suppression, as seen by disappearance of a storage protein subunit, was observed immediately upon formation of a wheat allohexaploid (genome BBAADD; see Galili and Feldman, 1984). Interestingly, suppression was reversible: the storage protein reappeared upon extraction of the tetraploid BBAA genomes. Similarly, attempts to transfer a leaf-rust resistance gene from tetraploid to hexaploid wheat failed because of a suppressor gene that was mapped to the D genome (Kerber and Green, 1980). Inter-genomic suppression of disease resistance genes is a common phenomenon as was noticed by us in several newly-formed allopolyploids (unpublished results). Another well-studied example of inter-genomic suppression is the silencing of rye ribosomal RNA genes in the presence of the wheat genome. Cytosine methylation is involved in this silencing as suggested by reactivation of the rye ribosomal RNA genes upon treatment with 5-aza-cytidine and by the use of methylation sensitive/insensitive isoschizomers (Houchins et al., 1997).

In addition to inter-genomic suppression, dosage compensation (non-linear gene dosage response) for storage proteins was also observed as an instant reaction to changes in doses from zero to six of the storage protein-coding chromosome (Galili et al., 1986). This is a common way to instantaneously reduce the negative effect of over-production and inefficiency of genes that exist in super-optimal dose. Novel variation in allopolyploids could also result through increased variation in dosage-regulated gene expression (Osborn et al., 2003). In this regard it is interesting to note that a novel phenotype is obtained in wheat when the *q* allele of hexaploid wheat exists in an extra dose; two doses of *q* determine a speltoid phenotype, i.e., non-free-threshing grains and long-tapering spikes, while five or six doses of this allele determine free-threshing grains and more compact and square-head spikes (Muramatsu, 1963).

Also, inter-genomic gene interactions may be, in some cases, expressed in novel traits that do not exist in their parental diploids. Some of these traits may have great adaptive value. Such interactions also have direct relevance to wheat cultivation. For example, the baking quality of bread wheat is due to the unique properties of its gluten – a product derived from the contribution of the three genomes of hexaploid wheat and thus exists only at the hexaploid level. In addition, the combination of a large number of spikelets per spike derived from *T. urartu* (the donor of the A genome), with several fertile florets per spikelet originating from the donors of the B and D genomes, facilitated the high fertility of durum and bread wheat. Similarly, the combination of cold hardiness of *Ae. tauschii* (the donor of the D genome) with the prolific nature of tetraploid wheat (the donor of the A and B genomes) enabled the expansion of wheat cultivation into colder regions. Likewise, enzyme multiplicity, derived from the activity of all homoeoalleles, has increased the ability of the allopolyploid to adapt to a wider range of environments. This might account for the very wide distribution of wheat under cultivation – much wider than that of any other cultivated plant.

Alterations in cytosine methylation (demethylation or new methylation) were found in newly synthesized allopolyploid wheat (Liu et al., 1998b; Shaked et al., 2001). Alterations in methylation patterns affected about 13% of a random set of genomic loci, both repetitive and low-copy sequences (Shaked et al., 2001). In a recent analysis of the transcriptome of a newly synthesized allotetraploid wheat, Kashkush et al. (2002) found

Table 3. Evolutionary changes (occurring during the life of the allopolyploid)

	Genome level	Gene level
Genetic:	Inter-genomic horizontal transfer of chromosomal segments	Gene inactivation through mutations, insertions and deletions
	Introgression of chromosomal segments from other allopolyploids or diploids and production of recombinant genomes	Functional diversification of homoeoalleles through mutations

that about 2% of the genes showed an altered expression. Transcript disappearance was caused either by gene loss or gene silencing. Southern blot analysis showed that inactivation in the amphiploid resulted from either methylation or elimination of the genes. cDNA-AFLP gels also revealed several cDNAs that were expressed only in the allopolyploids and not in the diploid progenitors. These included new transcripts corresponding to retrotransposon-like sequences (Kashkush et al., 2002). Similar studies (He et al., 2003) showed that the expression of a significant fraction of genes was altered in the synthetic hexaploid *T. turgidum-Ae. tauschii*. Most of these genes appeared to be silent while others were activated. Gene silencing was not due to chromosome or DNA loss but rather to gene regulation (He et al., 2003). The transcriptional activation of the retrotransposons was not associated with a burst of transposition, but resulted in suppression or activation of adjacent genes (Kashkush et al., 2003). For example, upon activation of the retrotransposon Wis 2-1A, its long terminal repeats drove the readout synthesis of new transcripts from adjacent sequences including antisense and sense strands of known genes. The activation of these antisense or sense transcripts was associated with silencing or activation of the corresponding genes, respectively (Kashkush et al., 2003). Yet, it is not possible, at this stage, to classify the genes that were inactivated or activated in the allopolyploids in rational groups. Activation of transposons due to allopolyploidization was observed also in other plants. Josefsson et al. (in press) found that newly formed *Arabidopsis* allopolyploids activate multiple transposons that remodel the genome.

A recent study in allopolyploid cotton (Adams et al., 2003) describes functional diversification of duplicated genes (sub-functionalization), i.e., differential expression of homoeoalleles in different tissues and in different developmental stages. Since functional differentiation of homoeoalleles is regulated by epigenetic changes, it is likely to occur immediately after allopolyploidization (Adams et al., 2003). Sub-functionalization, being an important aspect of allopolyploidy, was not yet studied in allopolyploid wheat.

From these studies it was concluded that allopolyploidization triggers gene silencing, gene elimination and gene activation via genetic and epigenetic alterations immediately upon allopolyploid formation. Gene activation is less frequent and involves mostly transposons. All the above rapid and reproducible genetic and epigenetic responses to allopolyploidization emphasize the plasticity of the wheat genomes. It is tempting to speculate that such plasticity is exploited to facilitate the establishment of the new species.

Evolutionary changes

The main evolutionary changes (Table 3) are also expressed at the structural and functional levels.

Structural changes

Events, such as inter-genomic horizontal transfer of chromosomal segments, repetitive sequences, transposons or genes between the constituent genomes, can occur almost exclusively in an allopolyploid background. These events may occur sporadically throughout the history of the allopolyploid species. Inter-genomic translocations, that are population or biotype specific, are widespread in allopolyploid wheat (Maestra and Naranjo, 1999). Invasion of the A genome by sequences from the B genome was detected in wild tetraploid wheat using GISH (Belyayev et al., 2000). The time course of such invasion is not known. The underlying mechanism might be inter-genomic transposition or concerted evolution. The possibility of inter-genomic transfer adds to the allopolyploid genomes' plasticity and enables the creation of new genetic combinations that are beyond the addition of two genomes.

Another important feature of evolution through allopolyploidy is that the allopolyploid condition facilitates hybridization and introgression between different allopolyploid species leading to the production of recombinant genomes that barely can be formed at the diploid level. Examples for such introgression between allotetraploid *Aegilops* species that share one genome and differ in the other genome(s), was provided by Zohary and Feldman (1962). Such hybridizations are eased by the shared genome, which acts as a buffer and ensures some fertility in the resulting hybrids. In such hybrids the two differentiated genomes, brought together from different parents, may exchange genetic material and recombine. Additional evidence for the existence of introgressed genomes in allopolyploid *Aegilops* were obtained recently from C-banding analysis (Badaeva et al., 2004). Also, introgression of a DNA sequence from allopolyploid wheat to *Ae. peregrina* was recently described by Weissmann et al. (2003).

The occurrence of a modified genome, consisting of the contribution of two or more diploid genomes, side by side with an unchanged one, is characteristic of the allopolyploid species of *Aegilops* and *Triticum*. This genomic structure facilitates continuous hybridization between allopolyploid species and further exchange of genetic material between the various modified genome(s). These dynamic genetic interconnections have led to the buildup of a wealth of genetic variability. Since many of these allopolyploid species tend to grow in mixture, the dy-

namic genetic interconnections render the mixed allopolyploid populations an active center of evolution.

In summary, in contrast to diploids, which are genetically isolated from each other and have undergone divergent evolution, allopolyploids of the genera *Triticum* and *Aegilops* exhibit convergent evolution because they contain genetic material from two or more different diploid genomes and can exchange genes with each other via hybridization or introgression, resulting in numerous genomic recombinations. This extensive inter-allopolyploid gene exchange, coupled with the breeding system of predominant self-pollination, is largely responsible for the wide variation ranges marking these allopolyploids and thus, for their apparent evolutionary success (Zohary and Feldman, 1962). Allopolyploidy is therefore of evolutionary importance here mainly because it has facilitated the formation of a superstructure that combines various genetic materials of the isolated diploids, allowing them to recombine.

Functional changes

The allopolyploid background per se, i.e., the presence of triplication of the genetic material, has relaxed constraints on the function of the multiple genes enabling, in the long term, genomic changes (continued genetic diploidization) such as silencing of one of the duplicated genes or divergence of one homoeologous locus to a new function. Thus, the accumulation of genetic variation through mutations or hybridizations is tolerated more readily in allopolyploid than in diploid species. In support of this hypothesis are theoretical considerations, such as a lack of selection against mutations due to the presence of duplicated or triplicated loci in the allopolyploids and experimental data showing resistance of allohexaploid wheat to irradiation compared to their diploid progenitors (Mac Key, 1954; Sears, 1972).

Most gene loci exist in triplicate dose in allohexaploid wheat. These homoeologous loci may differ from one another by allelic variations (homoeoalleles). Expression of all homoeoalleles coding for the same or similar functional proteins may increase the spectrum of the produced isozymes, and in case of multimeric enzymes, may lead to the formation of novel "hybrid" enzymes, resulting in greater physiological versatility and wider adaptability (Feldman et al., 1986). In support of this assumption are the reports of Mitra and Bhatia (1971) and Hart (1987) that hexaploid wheat expresses nearly all of the enzymes specified by the different homoeoalleles. Hence, maintenance of the activity of favorable homoeoallelic combinations confers obvious advantages. On the other hand, activity of all the homoeoalleles coding for non-functional proteins, e.g., storage protein genes, rRNA genes, tRNA genes and various structural protein genes, might be redundant and result in over-production and inefficiency. No wonder therefore, that homoeoalleles of these genes become inactive. Inactivation is brought about sporadically over a long time period during the life of the allopolyploid species by base substitution, insertion or deletion (Feldman et al., 1997). For instance, several lines of wild allotetraploid wheat have four active loci, two of the A genome and two of the B genome, for high molecular weight glutenins, other lines have three such loci, and still others have only two or one, indicating sporadic inactivation during the life of the species. Inactivation of these glutenin genes, as well as that of rRNA genes, is a non-random process (Feldman et al., 1986). Thus, allopolyploid wheat may possess two types of contradicting regulatory processes, maintenance of the activity of favorable homoeoallelic combinations and inactivation of the redundant ones.

Another interesting question is whether evolutionary processes that normally occur in diploids, such as microsatellite expansion, insertions, and point mutations, occur at a faster rate in allopolyploids compared to their diploid progenitors. By now, there is no direct evidence that diploid-like evolutionary processes are accelerated as a result of allopolyploidy. Transposon-induced mutations could also contribute to genetic diploidization by knocking out redundant genes, e.g. the recent Wis-2 retroelement insertion in a bread wheat glutenin gene (Harberd et al., 1987). However, there is no evidence for a transposition burst in allopolyploid wheat as a result of allopolyploidization. In cotton, on the other hand, there is evidence for inter-genomic spread of repetitive sequences after allopolyploid formation (Zhao et al., 1998). Overall, there is a lack of direct experimental evidence for an acceleration of evolutionary processes such as point mutations, transposition and microsatellite expansion as a result of allopolyploidy.

Concluding remarks

Stebbins (1971, 1980) stated that while polyploidy has been of great importance for the origin of species it has contributed little to progressive evolution. He assumed that polyploids, after they have been formed, evolved more slowly than their diploid relatives. By this statement he did not take into consideration that hybridization and chromosome doubling create two genetic shocks to which the newly formed allopolyploids react in a burst of genomic changes that, among others, produce novel genetic variation, nor that the allopolyploid genetic system per se triggers and facilitates genomic alterations that are not feasible at the diploid level.

From this review of recent studies in wheat, and from recent studies in other allopolyploid species (Leitch and Bennett, 1997; Comai, 2000; Soltis and Soltis, 2000; Wendel, 2000; Pikaard, 2001; Levy and Feldman, 2002), it is obvious that while the formation of an allopolyploid species is accomplished in one step, its establishment in nature as a successful species involves several cardinal genomic changes, of which some are rapid and non-Mendelian in nature, occurring immediately after the formation of the F_1 hybrid or the allopolyploid (revolutionary changes), and others occur sporadically over a long time period during the life of the allopolyploid species (evolutionary changes). The revolutionary changes stabilize the newly formed allopolyploid, enable it to benefit from the advantages of the inter-genomic interactions that increase its fitness so that it can compete well with its parental species. The evolutionary changes contribute to the build up of genetic variability and thus, increased adaptability. The genetic structure of the allopolyploid wheat has allowed for these changes to occur.

The questions raised by these studies are: (1) What are the factors affecting the elimination of non-coding and coding

sequences? (2) What are the mechanisms involved in sequence elimination? (3) What are the adaptive values of the genomic changes (genetic and epigenetic ones)? (4) What characterizes genes that are activated, eliminated or inactivated after allopolyploidization; and (5) What are the possible roles of CSSs in homology recognition and pairing initiation at the beginning of meiosis. Moreover, an interesting possibility that should be tested is whether the polyploid background enables an acceleration of evolutionary processes that are also present in diploids, such as point mutations, transposition or instability, which may increase the variation on which natural selection can act upon.

Our finding in wheat of allopolyploidization-induced sequence elimination is presumably a common phenomenon. Leitch and Bennett (in press), determining the amount of DNA in a large number of diploid and polyploid plants, found that loss of DNA following polyploidy formation is a widespread phenomenon of considerable biological significance. On the other hand, there is evidence that allopolyploidization also induces rapid amplification of DNA sequences. For example, the highly repetitive sequence Spelt52 was amplified in wheat allopolyploids containing *Ae. speltoides* as one parent (Salina et al., 2000, 2004). An interesting outcome of these studies is that plant genomes do not have a "one-way ticket to genetic obesity" due to retroelement accumulation, as was suggested by Bennetzen and Kellogg (1997). Rather, genome evolution involves dynamic processes of genome expansion and contraction (Bennetzen, 2002; Wicker et al., 2003).

A fascinating and yet not understood possible cause for evolutionary success is that allopolyploid species seem pre-adapted to chromosome doubling because they deal so efficiently with the rather dramatic event of allopolyploidization. They achieve harmonization of gene expression on a genomic scale by regulation mechanisms leading to gene silencing, dosage compensation, or, on the other hand, to gene activation. All we know about these mechanisms is that sometimes methylation is involved. We do not know either what the key player genes are whose alteration in expression may contribute to an increased fitness in the allopolyploid. Finally, it will be of interest to find out why some genomic combinations are successful and others not. Is it related to the ability to react to the shock of allopolyploidization, or to a positive combining ability between genomes (the equivalent of heterosis between species)?

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